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The Chemical Isomerization of Lactose to Lactulose by Using Sodium Hydroxide as Batch Reaction

* T. K. Nahla
Department of Food Sciences, College of Agriculture, University of Baghdad, Iraq
E-mail: *nahla_tariq@yahoo.com

ABSTRACT
Lactulose is a synthetic and non-absorbable disaccharide sugar containing glucose and fructose units. It is used for the treatment of constipation and hepatic encephalopathy. The purpose of this experiment was to synthesized lactulose in the presence of sodium hydroxide as a catalyst. The evaluation of lactulose synthesis was carried out at different concentration of sodium hydroxide (1, 2 and 3M) and the study was also conducted at different temperatures (30, 50, 70, and 90 °C). In addition, the kinetics of lactose isomerization with respect to the time also studied. Finally, it was concluded that the lactulose formation was determined by spectrophotometric method. The lactulose isomerization increased about more than 30.9% at 90 °C in the presence of 1M sodium hydroxide and 20 % lactose concentration.

***Keywords**: Lactose, isomerization, lactulose, sodium hydroxide.*

1. INTRODUCTION
Lactulose is a synthetic sugar, which is not adsorbed by humans or rodents due to the lack of enzymes which catalyzes the disaccharide sugars. It can be generated by either alkaline isomerization of lactose via the Lobry de Bruyn - Alberda van Ekenstein rearrangement or by enzyme-catalyzed synthesis. Based on the first reaction, different process schemes for the preparation of lactulose have been developed. The enzymatic synthesis of lactulose can be carried out using different pathways with the transgalactosylation reaction being the most promising[1]. In addition, It is slightly sweeter than lactose and can be used as a partial sucrose substitute in some food products[2] and can also be used as a food supplement in pediatric diets for the development of functional foods and in geriatric medicine for some targeted populations with severe constipation syndrome[3]. Furthermore, Lactulose is also widely used as a statement in hepatic encephalopathy. Lactulose is recognized as a prebiotic and it is used in the form of syrup for the treatment of some intestinal disorders[2].

Lactulose is synthesized by alkaline isomerization of lactose, a process that normally produces lactulose in low yields due to numerous side reactions. Montgomery and Hudson synthesized lactulose with calcium hydroxide for the first time[4]. Since then many catalysts including sodium hydroxide[5-8], potassium hydroxide, sodium carbonate[5], magnesium oxide[9] and organic alkaline reagents such as tertiary amines[10] have been used. These processes generally produce a high level of lactulose degradation, which leads to the formation of a considerable percentage of difficult-to separate, colored by-products which lower the lactulose yield.

Another group of processes uses complexing reagents such as aluminates[13-16], which facilitate the reaction with a minimum of secondary reactions and result in a high yield of lactulose by eliminating lactulose from the reaction equilibrium mixture in the form of a complex.

The purpose of present work study to analyze the effect of different molar concentration of sodium hydroxide at different temperatures (30, 50, 70 and 90˚C) in different time intervals for the quantification of lactulose.

2. MATERIALS AND METHODS
Five hundred mL of 1M sodium hydroxide solution was prepared by weighing ~ 37g of sodium hydroxide in a 500-mL of volumetric flask by dissolving in distilled water. 25 g of pure lactose was added to 500 mL of 1Msodium hydroxide solution to give 5% lactose. The mixture was heated at 30, 50, 70 and 90 ºC by using a hot plate magnetic stirrer for 1, 2, 3, 4, 5, 6 and 7 hr. 50 mL of mixture was taken at the end of each period and the pH was adjusted to 7 by using 10% phosphoric acid. The sodium phosphate precipitation was separated by using centrifuge at 4,000 rpm for 10 min., the supernatant was collected and stored in the refrigerator. 2M and 3M sodium hydroxide batch reactions were achieved as above. Spectrophotometric method was used to determine the degree of lactose isomerization into lactulose**.** 50mg resorcinol was dissolved in limit quantity of HCl (4M) and the volume was up made to 10mL by 4M HCl to prepare Seliwanoff's reagent (0.05%). A standard curve of mg/mL lactulose versus the absorbance at wavelength 480 nm was prepared by mixing different volumes of 10% lactulose and 10% lactose.
Five mL of lactulose sample solution was mixed with 4 mL- of Seliwanoff's reagent (0.05%) in 10 mL- test tube and heated in a boiling water bath for 5 min. The reaction tube was cooled immediately to 37 ± 2ºC .The absorbance was measured at 480 nm. A blank was prepared by mixing 5 mL- distilled water and 4 mL- Seliwanoff's reagent. Similar procedure was applied with the sample solutions. The quantity of lactulose in each sample was calculated by using the standard curve[11-12].

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Lactulose in the isomerization samples of pure lactose from batch reaction was estimated by Spectrophotometer. All samples were treated with Seliwanoff's reagent. The absorbance of those samples were recorded and compared with standard curve of lactulose. The quantity of lactulose in each sample was calculated by using the standard curve.

3. RESULTS AND DISCUSSION

The conversion of lactose to lactulose using sodium hydroxide was followed by the rapid degradation of lactulose into galactose and isosaccharinic acids[5-7].During this degradation, a brown colored reaction mixture was observed. To minimize lactulose degradation, a small amount of catalyst was used. The lactulose concentration increased gradually with the time, reached to a maximum and then declined. This was due to the degradation of lactulose to galactose and other by-products.

As shown in Figs. 1, 2 and 3 there was a direct proportional relationship between lactulose formation and temperature by using 1M sodium hydroxide as alkaline catalyst in the isomerization reaction. Similar, relation was found between lactulose formation and the period of reaction. The direct proportional relationship was true till 2 h of isomerization reaction, but there was a gradual decreasing in lactulose percentages over the 2 h reaction. In temperature degree 90 °C of isomerization of lactose to lactulose by using 1M sodium hydroxide showed a high lactulose formation during the 2st h of reaction. After 2 hour of the isomerization, the percentage of lactulose formation were 30, 28.80, 30.94% (of the maximum amount of lactulose after 2 h) when using 5, 10 and 20 % lactose, respectively (Table 1, 2 and 3).

Table.1: The percentage of lactulose formation by using 1M sodium hydroxide and 5% lactose at 30°, 50°, 70° and 90°.

Temp.	30°	50°	70°
Time (hour)			
1	19.63	20.00	6.88
2	24.00	25.00	3.00
3	22.00	24.00	1.00
4	20.00	22.00	9.00
5	19.00	21.00	8.00

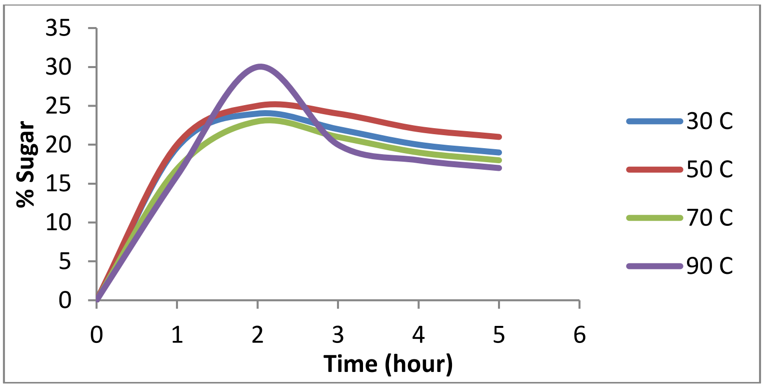

Fig.1: Effect of 1M sodium hydroxide on the formation of lactulose by using 5% lactose at 30°, 50°, 70° and 90°C.

Table. 2: The percentage of lactulose formation by using 1M sodium hydroxide and 10 % lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	12.50	12.44	14.06	15.00
2	14.38	13.00	26.25	28.80
3	12.60	12.00	25.00	27.00
4	12.00	11.00	20.00	22.00
5	11.00	10.00	15.94	19.00

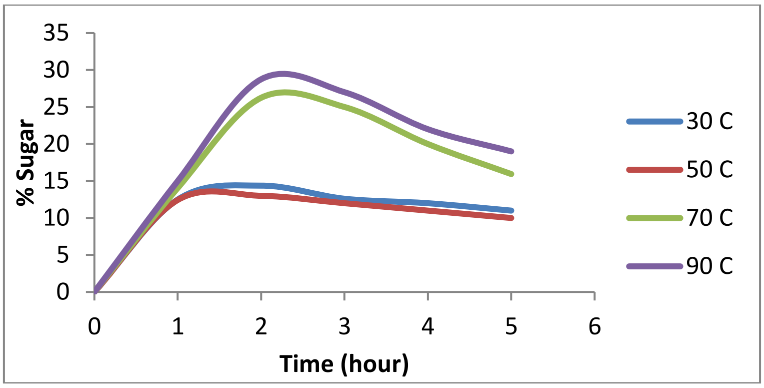

Fig.2: Effect of 1M sodium hydroxide on the formation of lactulose by using 10% lactose at 30°, 50°, 70° and 90°C.

Table.3: The percentage of lactulose formation by using 1M sodium hydroxide and 20 % lactose at 30°, 50°, 70° and 90°C

Temp.	30°	50°	70°	90°
Time (hour)				
1	14.06	8.59	12.97	14.75
2	10.90	9.06	28.24	30.94
3	10.40	9.22	15.78	18.28
4	9.69	9.00	14.00	17.34
5	9.22	9.00	14.00	15.15

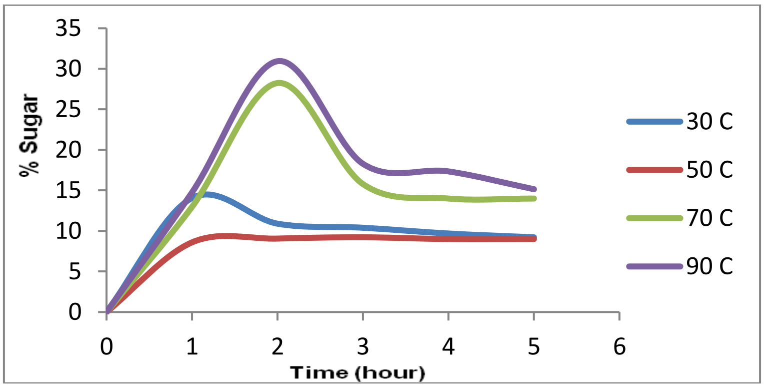

Fig.3: Effect of 1M sodium hydroxide on the formation of lactulose by using 20 % lactose at 30°, 50°, 70° and 90°C.

Figures 4, 5 and 6 show that the main quantity of lactulose produce during the first hour of isomerization reaction by using 2M sodium hydroxide as alkaline catalyst and the increasing was gradually ascending up to the 2 hr before it gradually decreased. Upon the 2nd h of the isomerization, the percentages of lactulose were 29.25, 28.75 and 30.47% of the higher percentages (after 2 h) of Lactulose when using 5, 10 and 20 % lactose at 90 ˚C, respectively. As compared with 1M, 2M sodium hydroxide, economically, gave insignificant increasing in lactulose formation especially after 2 h of reaction (Table 4, 5 and 6).

Table.4: The percentage of lactulose formation by using 2M sodium hydroxide and 5% lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	11.25	10.25	12.75	12.75
2	24.00	13.88	16.13	29.25
3	22.00	13.50	14.38	19.00
4	20.00	13.00	13.50	18.38
5	19.00	12.00	13.00	18.00

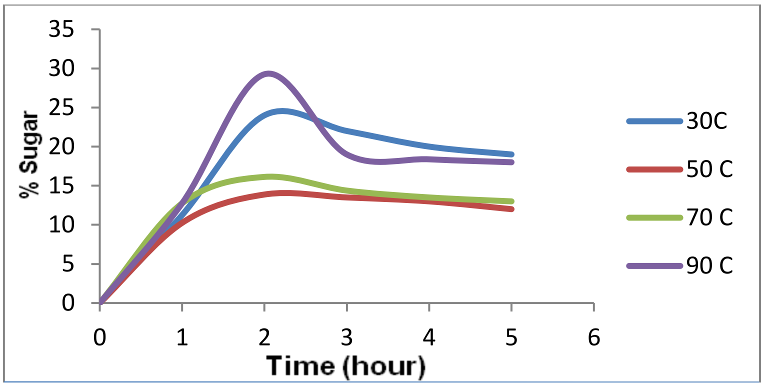

Fig.4: Effect of 2M sodium hydroxide on the formation of lactulose by using 5 % lactose at30°, 50°, 70° and 90°C.

Table.5: The percentage of lactulose formation by using 2M sodium hydroxide and 10 % lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	10.31	12.13	15.94	10.94
2	15.00	18.44	20.00	28.75
3	12.13	17.19	19.69	22.00
4	12.00	16.88	18.75	20.00
5	12.13	15.00	18.75	18.13

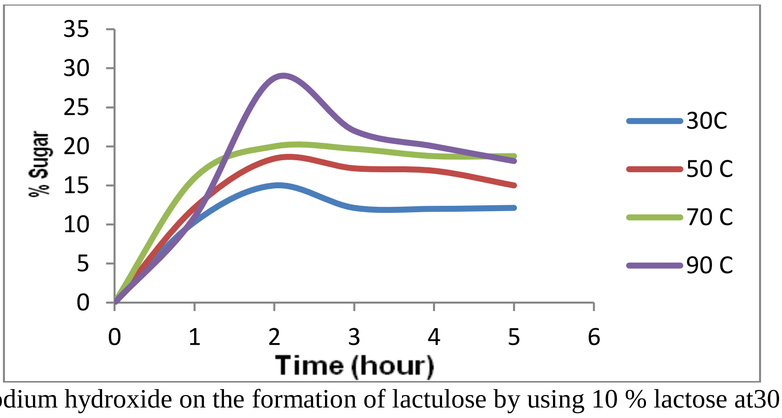

Fig.5: Effect of 2M sodium hydroxide on the formation of lactulose by using 10 % lactose at30°, 50°, 70° and 90°C.

Table.6: The percentage of lactulose formation by using 2M sodium hydroxide and 20 % lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	6.25	13.29	13.60	12.88
2	6.34	20.66	22.81	30.47
3	6.53	14.38	20.00	19.06
4	7.04	13.90	18.44	15.00
5	12.13	15.00	18.75	18.13

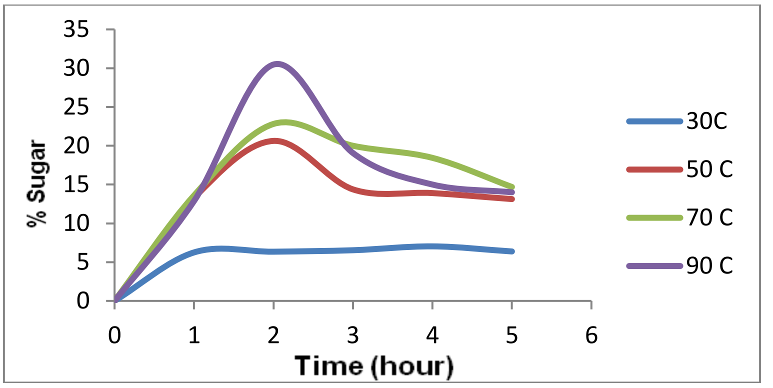

Fig. 6: Effect of 2M sodium hydroxide on the formation of lactulose by using 20 % lactose at 30°, 50°, 70° and 90°C.

The lactulose formation averages havebeen increased by using 3M sodium hydroxide as compared with 1 and 2M. Figs. 7, 8 and 9 show that there was a direct proportional relationship between the percentage of lactulose formation and the time of reaction. Again, the main quantity of lactulose was formed during the second hour of the isomerization 20.25, 23.44 and 24.00 % of the higher quantity of lactulose (after 2 hr.) was formed during one hour of isomerization reaction (Table 7, 8 and 9). A direct proportional relationship was found between galactose and fructose formations with both temperature and time, this result agreed with[1]who showed the initial time of reaction giving the main percentage of lactulose maximum quantity, that because there was not enough lactulose on the other side of the chemical equation to oppose the formation of more lactulose, as soon as the lactulose was accumulated with the by-products, the averages of lactulose formation going to be reduced.

As compared with 2M and 3M sodium hydroxide, economically, gave insignificant increasing in lactulose formation especially after two hour of reaction.

Table.7: The percentage of lactulose formation by using 3M sodium hydroxide and 5% lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	5.63	9.75	7.63	10.00
2	15.00	16.38	13.88	20.25
3	10.00	16.00	10.88	12.13
4	9.50	15.00	10.00	11.00
5	8.80	14.00	10.00	10.00

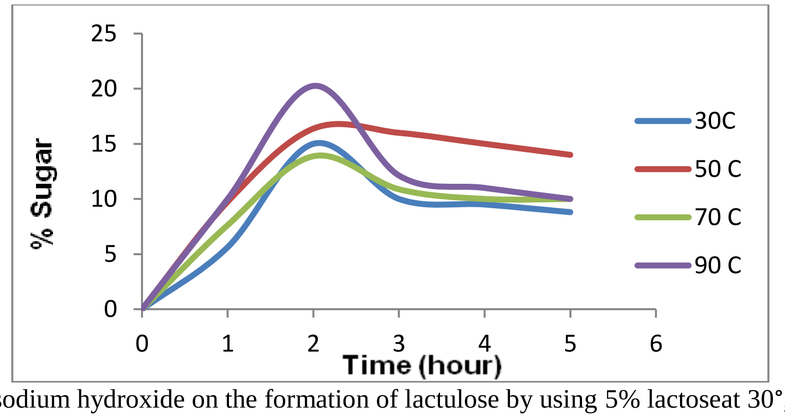

Fig. 7: Effect of 3M sodium hydroxide on the formation of lactulose by using 5% lactoseat 30°, 50°, 70°and90°C

Table.8: The percentage of lactulose formation by using 3M sodium hydroxide and 10 % lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	10.88	7.38	9.88	12.13
2	17.50	13.19	13.50	23.44
3	16.88	12.38	13.00	18.00
4	12.44	11.00	13.00	14.06
5	12.06	8.06	13.00	13.31

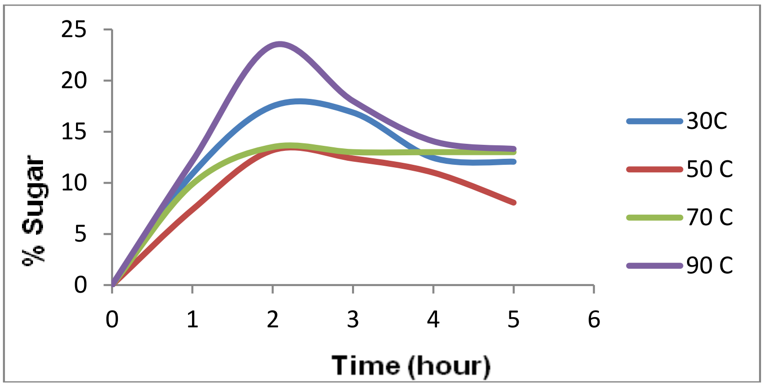

Fig. 8: Effect of 3M sodium hydroxide on the formation of lactulose by using 10 % lactose at30°, 50°, 70° and 90°C.

Table.9: The percentage of lactulose formation by using 3M sodium hydroxide and 20 %lactose at 30°, 50°, 70° and 90°C.

Temp.	30°	50°	70°	90°
Time (hour)				
1	6.25	12.81	12.34	17.50
2	5.88	18.13	22.34	24.00
3	6.31	17.50	22.00	21.40
4	6.00	16.25	20.72	18.50
5	6.00	16.00	17.19	17.90

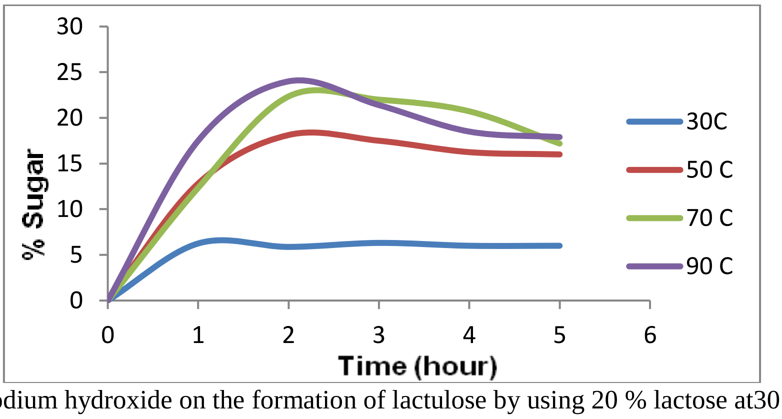

Fig.9: Effect of 3M sodium hydroxide on the formation of lactulose by using 20 % lactose at30°, 50°, 70° and 90°C.

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